

Analyzing Multiple Fluorobenzoate Tracers in the Presence of Interfering Compounds

William T. Stringfellow, Qinhong Hu, Robert TerBerg, and Grace Castro

Center for Environmental Biotechnology and Earth Sciences Division

E. O. Lawrence Berkeley National Laboratory

Berkeley, CA 94720

Summary

Fluorobenzoic acids (FBAs) are used as tracers in both vadose and saturated zone flow systems. Several chromatographic methods have been described for FBA analysis. Although these methods have been used effectively in a number of studies, they are subject to interference by nitrate, nitrite, chloride, bromide, and soluble organic matter. Consequently, current methods have significant limitations in field studies, especially at contaminated sites.

In this paper we describe and evaluate two HPLC methods for their ability to simultaneously analyze up to 14 FBAs in the presence of interfering compounds (chloride, nitrate and organic matter). The method using a pH 2.0 mobile phase (acid method) is generally superior to the method which uses a pH 2.9 mobile phase (buffer method) for the separation and analysis of multiple FBAs in the presence of interfering substances. The buffer method has one advantage of allowing a more rapid analysis of some FBAs in clean waters. The acid method is especially useful for samples containing high concentrations of nitrate and soluble organic matter.

Analytical problems in field studies can be minimized if methods and background interferences are fully evaluated prior to the collection of data. Resolution matrix tables are presented to assist in the selection of the proper analytical method and groundwater tracer combination for use in complex studies where interfering compounds are present. These resolution tables assist in the selection of FBA tracers that will not interfere with each other if used sequentially or in concert.

Introduction

Fluorobenzoic acids (FBAs) are being used as soil and water tracers in studies conducted at U. S. Department of Energy National Laboratories and other facilities (Hydro Geo Chem 1986, Boggs and Adams, 1992, Boggs et al. 1992, Bussod et al. 1998, Reimus and Turin 1997). The fluorobenzoates are attractive tracers because they do not occur naturally, are detectable at low concentrations, and are resistant to degradation (Bowman and Gibbens 1992). The transport behavior of FBAs is similar to bromide, a conservative tracer and can also have utility as reactive tracers (Benson and Bowman 1994, Bowman and Gibbens 1992, Jaynes 1994, Seaman 1998). The molecular size of FBAs are similar to many contaminants of interest, and FBAs have been used as models for organic contaminants in investigations of dispersion and diffusion mass transfer (Hu and Brusseau 1995).

FBAs can be used in both vadose and saturated zone flow systems. At experimental sites, such as Yucca Mountain, NV, the Waste Isolation Pilot Plant, NM, the Box Canyon Site, ID and Vadose Zone Observatory, CA, many tracer experiments are conducted one after another over a course of years. Studies may involve the use of multiple tracers including FBAs, dyes, microspheres, and bromide. Other sites, such as the Hanford Facility, may have high concentrations of nitrate, chloride, or soluble organic matter (SOM) in their groundwater and vadose zones. In all cases, it is important to develop methods for fluorobenzoate analysis that are able to distinguish a wide variety of FBAs from each other and that will allow the detection of FBA tracers at low concentrations in the presence of nitrate, chloride, and SOM.

The most widely used method for FBA analysis is an anion exchange method with UV detection developed by Bowman (1984). This method has been used effectively in a number of

studies and has been used to separate up to six FBAs simultaneously (Benson and Bowman 1994, Bowman and Gibbens 1992). Unfortunately, the method is subject to interference by nitrate, nitrite, bromide, and SOM (Benson and Bowman 1994, Bowman and Gibbens, 1992). Analysis can also be complicated by the rapid degradation of the column in the presence of the low pH elution buffer (TerBerg 1993). Field data has been lost at sites which have significant background nitrate and organic matter concentrations when this method was used (Boggs et al. 1992, Boggs and Adams 1992).

Other methods for analysis of FBAs include an ion chromatographic method with conductivity detection (Pearson et al. 1992) and a high performance liquid chromatography (HPLC) method developed for the analysis of pentafluorobenzoic acid, m-(trifluoromethyl)-benzoic acid, and benzoic acid (Stetzenbach et al. 1982). The method of Stetzenbach et al. (1982) enhanced the detection limit for the FBAs, but was only optimized for the separation of three compounds. The ion chromatography method of Pearson et al. (1992) has also been used to separate three FBAs in the same sample, but is subject to interference by chloride, a common groundwater constituent.

The objective of this study was to develop a reverse phase HPLC method for the analysis of FBA groundwater tracers that would be free of interference from polar ground water constituents such as nitrate and chloride. It was desired to develop methods that could be used to measure up to as many as fourteen different FBAs at the same time. In this paper, we present a detailed analysis of HPLC methods and discuss their use for the simultaneous measurement of multiple FBAs in the presence of interfering compounds. Resolution matrix tables are presented

to assist in the selection of groundwater tracers for use in complex studies where interfering compounds may be present.

Materials & Methods

Reagents:

Phosphoric acid (85%), monobasic potassium phosphate, potassium nitrate, sodium chloride, and acetonitrile were purchased from EM Science (Gibbstown, NJ). Water was 18.2 Mohm quality from a Millipore filtration system (Bedford, MA). FBAs were purchased from Aldrich Chemicals. 2,3,6-Trifluorobenzoic acid (2,3,6-TFBA), 2,4,5-trifluorobenzoic acid (2,4,5-TFBA), 2,3,4,5-tetrafluorobenzoic acid (2,3,4,5-TeFBA), 2,3,5,6-tetrafluorobenzoic acid (2,3,5,6-TeFBA), and pentafluorobenzoic acid (PFBA) were 99% purity. 2,3-difluorobenzoic acid (2,3-DFBA), 2,4-difluorobenzoic acid (2,4-DFBA), 2,5-difluorobenzoic acid (2,5-DFBA), 2,6-difluorobenzoic acid (2,6-DFBA), 3,4-difluorobenzoic acid (3,4-DFBA), 2,3,4-trifluorobenzoic acid (2,3,4-TFBA), 2,4,6-trifluorobenzoic acid (2,4,6-TFBA), and 3,4,5-trifluorobenzoic acid (3,4,5-TFBA) were 98% pure. 3,5-Difluorobenzoic acid (3,5-DFBA) was 97% pure. All compounds were used without further purification.

A pH 2.9 buffer was made by titrating 500 mL of a 0.06 Molar monobasic potassium phosphate solution (initial pH 4.5) with a 0.07 Molar phosphoric acid solution (initial pH 2.0). Acidified water (pH 2.0) and acidified acetonitrile were prepared by adding four mL of phosphoric acid to one liter of solvent (0.07 Molar solutions).

Experimental soluble organic matter (SOM) was prepared by placing 400 grams of potting soil in cheesecloth, simmering in one liter of distilled deionized water for 20 minutes and filtering the solution through a 0.2 micron filter.

HPLC Analysis:

High performance liquid chromatography (HPLC) separation was achieved using an Waters Alliance System HPLC equipped with a 996 photodiode array detector. The Alliance system consists of a refrigerated autosampler, pumps and a heated column chamber. Separations were conducted using a 150 mm x 4.6 mm, 5 μ m LC-PAH column with a C-18 guard column (Supelco, Bellefonte, PA) with a one mL per minute flow rate at 30° C. The LC-PAH column is a C-18 column with less cross-linking than a standard C-18 column for increased efficiency in the separation of planar molecules.

Two different methods were tested for the analysis of FBAs in water samples. FBAs were analyzed at 100 mg/L concentrations with 10 μ L injections. For the “buffer method,” the FBA tracers were separated by isocratic elution using a mobile phase of 35% (v/v) acetonitrile and 65% pH 2.9 phosphate buffer. For the “acid method,” separation was achieved using 25% acidified acetonitrile and 75% 0.07 Molar phosphoric acid. All FBAs were quantified by measuring their UV absorbance at 210 nm.

Calculations:

Resolution between peaks was quantified by calculating a resolution number (R_s):

$$R_s = |2(R_1 - R_2)/(B_1 + B_2)|$$

where R_1 is the retention time of analyte 1, R_2 is the retention time of analyte 2, B_1 is the peak base width for analyte 1 and B_2 is the peak base width of the peak for analyte 2 (Dolan and Snyder 1989). The base width of a given peak will depend on a number of factors including analyte residence time in the column. In our study, late eluting peaks at 100 mg/L concentrations of FBAs had peak widths less than 0.5 minutes. We took a 0.5 minute base width as a

conservative estimate that was used in all calculations of R_s for the FBAs. The quantification limit for the FBAs was calculated as five times background at 210 nm.

Results & Discussion

Separation of FBA tracers

The use of the buffer method and the acid method for the separation of fourteen FBAs was compared. The retention times for the FBAs using the buffer and acid methods are given in Tables 1 and 2, respectively. The elution order is sensitive to changes in pH. A lower pH promotes a stronger interaction between the FBAs and the column and the subsequently the retention times are longer with the acid method. Longer retention times results in greater compound resolution. Tables 1 and 2 present resolution numbers in relation to each other for the fourteen FBAs by the buffer and acid methods, respectively. These tables can be used to determine the compatibility of different FBAs for use in concert or in sequence and to evaluate the impact of interfering compounds on data collection.

The buffer method allows more rapid analysis of all FBAs tested if the compounds were being analyzed individually, but the acid method gives superior resolution of more compounds (Tables 1 and 2). For example, 2,4,6-TFBA will elute at 2.3 minutes by the buffer method, but will require a run time of greater than 7 minutes if analyzed by the acid method. However, using the buffer method for analysis would not be advisable if 2,4,6-TFBA were to be used at a study site where PFBA, 2,6-DFBA, 2,3,5,6-TeFBA, or 2,3,6-TFBA had been used previously. These compounds will interfere with the analysis of 2,4,6-TFBA by the buffer method, but not by the acid method (Tables 1 and 2).

Analysis of FBAs in the presence of interfering compounds

The ability of these methods to separate FBAs from interfering compounds was evaluated. Tables 3 and 4 present resolution numbers for the FBAs with nitrate and organic solutions. Chloride (R_t 1.61 minutes) does not significantly adsorb UV light at 210 nm and consequently does not interfere with the analysis of FBAs by either method.

Nitrate at 100 mg/L (as N) gave a very large peak with a retention time of 1.63 minutes with both methods. The nitrate peak essentially eluted with the solvent, or unretained peak (1.60 minutes). The peak had a base width of 1.2 minutes using the acid method and 0.65 minutes using the buffer method. High concentrations of nitrate will not interfere with the analysis of FBAs by the acid method (Table 4). However, using the buffer method, the first three compounds to elute, PFBA, 2,3,6-TFBA, and 2,3,5,6-TeFBA have a resolution number less than 1.0 with nitrate (Table 3). 2,6-DFBA has a resolution number of 1.04. This result suggests that if high nitrates are present it would be advisable to use the acid method to insure that nitrate will not interfere in the analysis.

Interference from SOM was investigated using a soil extract. By the buffer method, the SOM separated into two peaks at 1.61 minutes and 2.04 minutes. These peaks were reasonably narrow (base width of 0.68 and 0.53 minutes, respectively) and only interfered with the early eluting peaks 2,3,5,6-TeFBA, 2,3,6-TFBA, 2,4,6-TFBA, and PFBA (Table 3). With the acid method, the SOM exhibited four confluent peaks at 1.62, 1.78, 1.92 and 2.13 minutes. These peaks can be treated as one large peak with a retention time of 1.9 minutes and a base width of 3.7 minutes. With the acid method, the SOM does not interfere with the analysis of any FBA, because the first FBA elutes at 5 minutes (Table 4).

Quantification limits.

Both methods allow quantification of between 5 and 7 nanograms of FBA per injection by measuring UV absorbance at 210 nm. This yields an effective lower quantification limit of 0.07 ng/uL with a 100 uL injection. This is comparable to the anion method of Bowman (1984). Some FBAs have an optimum absorbance closer to 225 nm. For these compounds, a small increase in sensitivity can be obtained by measuring UV absorbance at the optimal absorbance value for each compound.

Summary & Conclusions

Currently available methods for FBA tracer analysis were determined to be inadequate for the separation of multiple traces in the presence of interfering compounds, particularly nitrate. Two HPLC methods for FBA analysis were developed and found to be useful for the measurement of multiple FBAs in the presence of interfering substances.

The acid method (pH 2.0) is generally superior to the buffer method (pH 2.9) for the separation and analysis of multiple FBAs in the same sample and in the presence of interfering substances. The acid method is especially useful for samples containing high concentrations of nitrates. Organic matter from soil extracts were not found to interfere with FBA analysis by the acid method. However, highly polluted water or water with high organic content could potentially contain compounds that will interfere with FBA analysis by the acid method. It is prudent in any case to pre-screen background samples to determine if interfering organic compounds are present that will elute after 5.0 minutes. Compounds that elute after 5 minutes by the acid method are more likely to interfere with FBA analysis. The buffer method has the one advantage of allowing a more rapid analysis of selected FBAs in clean waters.

Analytical problems in field studies can be minimized if methods are fully evaluated prior to the collection of data. Care should be taken in selecting tracers to insure that sequential studies do not interfere with each other. Tables 1 and 2 can be used to assist in the selection of FBA tracers that will not interfere with each other if used sequentially or in concert. Prescreening background samples for interfering compounds can help in the selection of a robust method for the analysis of tracers under field conditions. Resolution number matrixes, such as shown in Tables 3 and 4, can serve as useful guides for the selection of methods and tracers for use in complex studies.

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Table 1: Buffer method resolution number matrix. Numbers equal or greater than 1.0 indicate that analytes will not interfere with each other.

	R_t^1	2,3- DFBA	2,3,4- TFBA	2,3,4,5- TeFBA	2,3,5,6- TeFBA	2,3,6- TFBA	2,4- DFBA	2,4,5- TFBA	2,4,6- TFBA	2,5- DFBA	2,6- DFBA	3,4- DFBA	3,4,5- TFBA	3,5- DFBA
2,3- DFBA	3.42	0.0												
2,3,4- TFBA	4.13	1.4	0.0											
2,3,4,5- TeFBA	4.05	1.3	0.2	0.0										
2,3,5,6- TeFBA	2.14	2.6	4.0	3.8	0.0									
2,3,6- TFBA	2.07	2.7	4.1	4.0	0.1	0.0								
2,4-DFBA	3.68	0.5	0.9	0.7	3.1	3.2	0.0							
2,4,5- TFBA	3.87	0.9	0.5	0.4	3.5	3.6	0.4	0.0						
2,4,6- TFBA	2.33	2.2	3.6	3.4	0.4	0.5	2.7	3.1	0.0					
2,5-DFBA	3.28	0.3	1.7	1.5	2.3	2.4	0.8	1.2	1.9	0.0				
2,6-DFBA	2.23	2.4	3.8	3.6	0.2	0.3	2.9	3.3	0.2	2.1	0.0			
3,4-DFBA	4.95	3.1	1.6	1.8	5.6	5.8	2.5	2.2	5.2	3.3	5.4	0.0		
3,4,5- TFBA	5.80	4.8	3.3	3.5	7.3	7.5	4.2	3.9	6.9	5.0	7.1	1.7	0.0	
3,5-DFBA	4.71	2.6	1.2	1.3	5.1	5.3	2.1	1.7	4.8	2.9	5.0	0.5	2.2	0.0
PFBA	2.00	2.8	4.3	4.1	0.3	0.1	3.4	3.7	0.7	2.6	0.5	5.9	7.6	5.4

¹R_t = retention time, R_t for unretained peak is 1.60 minutes.

Table 2: Acid method resolution number matrix. Numbers equal or greater than 1.0 indicate that analytes will not interfere with each other.

	R_t¹	2,3- DFBA	2,3,4- TFBA	2,3,4,5- TeFBA	2,3,5,6- TeFBA	2,3,6- TFBA	2,4- DFBA	2,4,5- TFBA	2,4,6- TFBA	2,5- DFBA	2,6- DFBA	3,4- DFBA	3,4,5- TFBA	3,5- DFBA
2,3- DFBA	7.53	0.0												
2,3,4- TFBA	11.46	7.9	0.0											
2,3,4,5- TeFBA	16.80	18.5	10.7	0.0										
2,3,5,6- TeFBA	6.74	1.6	9.4	20.1	0.0									
2,3,6- TFBA	6.18	2.7	10.6	21.2	1.1	0.0								
2,4-DFBA	7.38	0.3	8.2	18.8	1.3	2.4	0.0							
2,4,5- TFBA	9.82	4.6	3.3	14.0	6.2	7.3	4.9	0.0						
2,4,6- TFBA	6.77	1.5	9.4	20.1	0.1	1.2	1.2	6.1	0.0					
2,5-DFBA	6.85	1.4	9.2	19.9	0.2	1.3	1.1	5.9	0.2	0.0				
2,6-DFBA	5.14	4.8	12.6	23.3	3.2	2.1	4.5	9.4	3.3	3.4	0.0			
3,4-DFBA	11.53	8.0	0.1	10.5	9.6	10.7	8.3	3.4	9.5	9.4	12.8	0.0		
3,4,5- TFBA	17.93	20.8	12.9	2.3	22.4	23.5	21.1	16.2	22.3	22.2	25.6	12.8	0.0	
3,5-DFBA	11.65	8.2	0.4	10.3	9.8	10.9	8.5	3.7	9.8	9.6	13.0	0.2	12.6	0.0
PFBA	9.50	3.9	3.9	14.6	5.5	6.6	4.2	0.6	5.5	5.3	8.7	4.1	16.9	4.3

¹R_t = retention time, R_t for unretained peak is 1.60 minutes.

Table 3: Buffer method resolution number matrix for interfering compounds. Numbers equal or greater than 1.0 indicate that compounds will not interfere with the analysis of the FBA.

	R_t^1	2,3- DFBA	2,3,4- TFBA	2,3,4,5- TeFBA	2,3,5,6- TeFBA	2,3,6- TFBA	2,4- DFBA	2,4,5- TFBA	2,4,6- TFBA	2,5-DFBA 2,6-DFBA	3,4- DFBA	3,4,5- TFBA	3,5- DFBA	PFBA
Nitrate	1.63	3.11	4.35	4.21	0.89	0.77	3.57	3.90	1.22	2.87	1.04	5.77	5.36	0.64
SOM ² peak 1	1.61	3.06	4.26	4.13	0.89	0.77	3.50	3.82	1.21	2.82	1.04	5.65	5.25	0.65
SOM peak 2	2.04	2.68	4.06	3.90	0.19	0.06	3.18	3.55	0.56	2.41	0.37	5.65	5.18	0.08

¹ R_t = retention time, R_t for unretained peak is 1.60 minutes.

² SOM, soluble organic matter

Table 4: Acid method resolution number matrix for interfering compounds. Numbers equal or greater than 1.0 indicate that compounds will not interfere with the analysis of the FBA.

	R_t¹	2,3- DFBA	2,3,4- TFBA	2,3,4,5- TeFBA	2,3,5,6- TeFBA	2,3,6- TFBA	2,4- DFBA	2,4,5- TFBA	2,4,6- TFBA	2,5-DFBA	2,6-DFBA	3,4- DFBA	3,4,5- TFBA	3,5- DFBA	PFBA
Nitrate	1.63	6.95	11.58	17.86	6.02	5.36	6.78	9.65	6.06	6.15	4.14	11.66	19.19	11.80	9.27
SOM¹	1.90	2.68	4.55	7.10	2.30	2.04	2.61	3.77	2.32	2.36	1.54	4.59	7.63	4.64	3.62

¹ R_t = retention time, R_t for unretained peak is 1.60 minutes.

² SOM, soluble organic matter.